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Original article

Iron deficiency and hematological changes in adult patients after Fontan operation

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ABSTRACT

Background: Growing evidence indicates that iron-deficiency anemia is common in patients with congenital heart diseases.

The aim of this study was to characterize hematologic changes and iron metabolism in adult Fontan patients. We also searched for the associations between these parameters and physical performance in the study group.

Methods and results: Thirty-two white Fontan patients with a mean age of 25 ± 4.5 years and 30 healthy control subjects matched for age and sex were studied. Complete blood count together with iron-related parameters was determined in plasma of peripheral venous blood. The cardiopulmonary exercise test was performed.

The Fontan patients had higher red blood cell counts (6.0 ± 2.1 × 10^9/μl vs. 4.8 ± 0.4 × 10^9/μl, $p < 0.001$), hemoglobin (16.7 ± 1.4 g/dl vs. 14.2 ± 1.3 g/dl, $p < 0.001$), hematocrit (49 ± 3.4% vs. 42.1 ± 3.1%, $p < 0.001$), red cell distribution width (RDW) (14.3 ± 2.4% vs. 12.8 ± 0.5%, $p < 0.001$), while mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were similar in both the groups. Compared to the controls, the Fontan patients had higher unsaturated iron binding capacity (46.1 ± 12.6 μmol/l vs. 38.4 ± 11.9 μmol/l, $p = 0.02$), total iron-binding capacity (62.8 ± 9.8 μmol/l vs. 57.8 ± 8.5 μmol/l, $p = 0.04$), lower transferrin saturation (27.4 ± 11.4% vs. 34.6 ± 13.4%, $p = 0.03$), and oxygen uptake, while iron and ferritin levels were comparable in both the groups. The multivariate model showed that $SatO_2$ and cystatin C were independent predictors of RDW, and alanine aminotransferase was an independent predictor of ferritin level. Interestingly RDW was an independent predictor of oxygen uptake.

Conclusion: Adult patients after Fontan operation despite having increased hemoglobin, hematocrit, and red blood cells have insufficient iron stores. Red cell distribution width is an indicator of iron deficiency in adult Fontan patients and it correlates with lower *exercise capacity*. Elevated ferritin levels in adult patients after Fontan surgery are associated with liver failure.

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Introduction

The Fontan procedure, described for the first time in 1971, is presently a widely accepted method of treating patients with single ventricle heart [1]. The absence of the ventricle that pumps blood to the pulmonary bed results in restricted pulmonary venous return, which in turn causes a decrease in systemic ventricle preload [2]. Some patients demonstrate a gradual decrease in arterial blood saturation, increased hematocrit (HCT) values, and cyanosis. This is

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associated with the presence of abnormal arterial-venous fistulas in the pulmonary bed, intrahepatic venous shunts, increased pulmonary resistance, atrioventricular valve regurgitation, impaired systolic and diastolic function of the systemic ventricle, and intracardiac shunts, e.g. fenestration [3]. Earlier reports described the prevalence of cyanosis in adult patients after the Fontan procedure as 20–25% [4,5]. Erythrocytosis is a physiological increase in red cell number as a consequence of chronic hypoxia. This is a compensatory mechanism, leading to increase in tissue oxygen supply [6].

Iron-deficiency anemia is the most common type of anemia; it may result from blood loss, diet iron deficiency, abnormal absorption, and increased requirements. Hemoglobin (HGB) concentration is the most reliable indicator of anemia at the population level [7,8]. Previous investigations indicated high frequency of anemia in patients with congenital cyanotic heart defects [9]. More than one-third of patients with congenital cyanotic heart defects are believed to be iron deficient [10]. Collins et al. [11] reported that anemia is common in patients with complex congenital heart diseases and ventricular dysfunction, in particular those with Fontan physiology. Moreover, anemia is associated with increased mortality rates in this group of patients [10]. Numerous studies on anemia in patients with congenital heart defects and heart failure were performed in subjects with biventricular hearts [7,11–15]. The cause of anemia in adult patients after Fontan operation remains unclear, and its influence on exercise capacity has not been examined. To the best of our knowledge, there are few investigations addressing the assessment of hematological and iron-related parameters in adult Fontan patients.

The aim of this study was to characterize hematologic changes and iron metabolism in adult Fontan patients. We also searched for the associations between these parameters and physical performance in the study group.

Materials and methods

Study participants

All the study patients were recruited consecutively at the John Paul II Hospital in Krakow, Poland, between August 2012 and December 2012. Patients were eligible if they were aged ≥18 years, had Fontan physiology, and were in stable clinical condition for at least 3 months before enrollment to the study. The main exclusion criteria were renal insufficiency, neoplasmatic diseases, infection, inflammation, major trauma within the past three months, pregnancy, diabetes, and alcohol abuse. In addition, 30 age- and sex-matched healthy volunteers were recruited. None of the participants were on iron supplementation or were phlebotomized.

Study protocol

Clinical and demographic variables were extracted from the patients' medical records. In every patient we assessed laboratory parameters, body mass index, arterial oxygen saturation, and ejection fraction of the systemic ventricle. Body mass index was calculated as mass (kg)/height (m)^2. Ejection fraction of the single ventricle (EFSV) was assessed using the Simpson method (Vivid 7, GE Medical Systems No 4183V7, Milwaukee, WI, USA). Cardiopulmonary exercise test (CPET) was performed using a modified Bruce protocol (ZAN-600 type USB No. 6660161). During the test, electrocardiogram, blood pressure, clinical symptoms, and duration of exercise were recorded. The monitored gas parameters included exercise time (*T*, min), peak oxygen uptake (VO_{2peak}, ml/kg/min and %predicted value) and oxygen uptake at anaerobic threshold (VO_{2AT}, %predicted value). Oxygen saturation ($SatO_2$, %) was measured by pulse oximetry in the room air.

The study protocol was approved by the local Ethical Committee and each subject granted informed consent to participate in the investigation.

Laboratory investigations

Blood samples were collected from the antecubital vein between 07:00 h and 09:00 h, after overnight fasting for at least 12 h. White blood cells (WBC), platelet count, red blood cells (RBC), HCT, HGB, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count, total protein, alanine aminotransferase (ALT), creatinine, gamma-glutamyltranspeptidase (GGTP), alkaline phosphatase (ALP), C-reactive protein (CRP), uric acid, creatinine, estimated glomerular filtration rate (eGFR), cystatin C, N-terminal pro-B-type natriuretic peptide (NT-proBNP), bilirubin, and international normalized ratio (INR) were assayed using routine laboratory techniques. Hyaluronic acid (HA) levels were measured with a commercial enzyme-linked immunosorbent assay (ELISA) kit (R&D systems, Minneapolis, MN, USA) according to the manufacturer's instructions. This sandwich assay uses human recombinant aggrecan as the immobilized capture phase for HA in the subject's serum sample and is designed to measure >35 kDa molecules of HA. Mean intra-assay coefficient of variation was 5.9%. Iron concentration values (normal range: men 6.6–26 μmol/l, women 11–28 μmol/l) and unsaturated iron-binding capacity (UIBC) (normal range: 20–62 μmol/l) were determined by the colorimetric method. Total iron-binding capacity (TIBC) (normal range: 45–70 μmol/l) was calculated as TIBC (μmol/l) = Iron (μmol/l) + UIBC (μmol/l). Transferrin saturation (TSAT) (normal range: 20–40%) was calculated as TSAT (%) = iron (μmol/l)/TIBC (μmol/l) × 100. Ferritin (normal range: 30–400 μg/l) was determined by the latex particle-enhanced immunoturbidimetric method (Cobas 6000, Roche, Rotkreuz, Switzerland). Anemia was defined as HGB concentration <13 g/dl in males and <12 g/dl in females [9].

Statistical analysis

Continuous variables are expressed as mean ± SD. Categorical variables are described as counts and percentages. Patients after Fontan operation and controls were compared using the Mann–Whitney *U*-test for the continuous variables and with the chi square test for categorical variables. Correlations between the individual parameters were calculated using the Spearman rank test. Factors that determine RDW, ferritin level, and VO_{2peak} were analyzed using multiple logistic regression analysis. A *p*-value <0.05 was considered statistically significant. The statistical analyses were performed with the PQStat version 1.4.2.324 software (Poznań/Plewiska, Poland).

Results

Group characteristics

We enrolled 32 Fontan patients (11 men, 20 women) aged 25 ± 4.5 years. The mean age at Fontan operation was 5.3 ± 3.5 (2–14) years. The mean follow-up time was 19.6 ± 5.2 (15–30) years. Underlying cardiac malformations were as follows: 14 (44%) patients with tricuspid atresia, 10 (31%) patients with pulmonary atresia and ventricular septal defect, two (6%) patients with double outlet right ventricle and left ventricular hypoplasia, and six subjects (19%) with right ventricular hypoplasia. Modification of the Fontan operation included direct right atrium–pulmonary artery connection in four (12%) patients and total cavopulmonary connection (TCPC) by means of intraatrial lateral tunnel in the remaining

28 (88%) patients. The fenestration was present in 10 (31%) TCPC patients. Majority of the patients (30, 94%) had single ventricle of the left ventricular morphology and the remaining two (6%) had morphologically right systemic ventricle. Seven (22%) patients were in New York Heart Association (NYHA) class I, 22 (69%) in NYHA class II, and three patients (9%) in NYHA class III. Ten (32%) patients were treated with warfarin, two (6%) with enoxaparin, and 15 (47%) with aspirin.

The Fontan patients did not differ from the controls with regard to age, sex, and BMI. The results are presented in Table 1. Compared to the controls, the Fontan patients had lower EFSV (51.1 ± 5.6% vs. 69.6 ± 5.4%, $p < 0.001$), $SatO_2$ (87.6 ± 7% vs. 96.9 ± 1.5%, $p < 0.001$), VO_{2peak} (23.7 ± 6.4 ml/kg/min vs. 49.3 ± 8.4 ml/kg/min, $p < 0.001$ and 61.4 ± 16.8% vs. 121.6 ± 19.6%, $p < 0.001$), VO_{2AT} (46.2 ± 16.2% vs. 55.7 ± 7%, $p = 0.005$), T (15.3 ± 2.7 min vs. 17 ± 2.5 min, $p = 0.01$), and higher ALT (28.5 ± 12.1 IU/l vs. 21.5 ± 6.1 IU/l, $p = 0.007$), cystatin C (1.0 ± 0.3 mg/l vs. 0.84 ± 0.1 mg/l, $p = 0.01$), bilirubin level (21.7 ± 15.1 μmol/l vs. 9.2 ± 4.6 μmol/l, $p < 0.001$), GGTP (75.7 ± 46.0 U/l vs. 19.3 ± 9.6 U/l, $p < 0.001$), ALP (67.4 ± 21.2 U/l vs. 51.2 ± 16.6 U/l, $p < 0.001$), INR (1.2 ± 0.2 vs. 1 ± 0.1, $p < 0.001$), HA (65.8 ± 59.3 ng/ml vs. 27.0 ± 23.3 ng/ml, $p < 0.001$) as well as NT-proBNP (320.1 ± 544 pg/ml vs. 54.3 ± 26.9 pg/ml, $p = 0.009$). There were no differences in total protein, creatinine, eGFR, and CRP levels. We have not diagnosed anemia in any Fontan patients. Among 32 analyzed patients low iron values were noted in four (12%), of which two (6%) presented with symptoms of protein-losing enteropathy manifested as edema of the lower extremities and ascites.

Hematologic alterations

The Fontan patients had higher RBC ($6.0 \pm 2.1 \times 10^9$/μl vs. $4.8 \pm 0.4 \times 10^9$/μl, $p < 0.001$), HGB (16.7 ± 1.4 g/dl vs. 14.2 ± 1.3 g/dl, $p < 0.001$), HCT (49 ± 3.4% vs. 42.1 ± 3.1%, $p < 0.001$), and RDW (14.3 ± 2.4% vs. 12.8 ± 0.5%, $p < 0.001$), while MCV, MCH, and MCHC were similar in both the groups. The results are shown in Table 2. There were no correlations between $SatO_2$ and RBC, HCT, and HGB. Interestingly, seven (22%) Fontan patients had RDW above the normal range (14.5%). RDW correlated negatively with $SatO_2$ ($r = -0.49$; $p = 0.008$), SVEF ($r = -0.52$, $p = 0.02$), ALT ($r = -0.4$, $p = 0.02$), MCH ($r = -0.48$, $p = 0.04$), iron ($r = -0.49$, $p = 0.05$), TSAT ($r = -0.4$, $p = 0.04$), and VO_{2peak} ($r = -0.5$, $p = 0.001$) and positively with CRP ($r = 0.4$, $p = 0.04$) and with follow-up time ($r = 0.4$, $p = 0.04$). The results are shown in Figs. 1 and 2. There was no correlation between RDW and NT-proBNP.

Table 1
Baseline clinical and laboratory characteristics of Fontan patients and controls.

	Fontan $N = 32$	Controls $N = 30$	p
Age (years)	24.9 ± 6	25.4 ± 5.7	0.09
Male, *n* (%)	20 (66)	22 (69)	0.7
BMI (kg/m^2)	22 ± 1.8	21.7 ± 2.6	0.2
SVEF (%)	51.1 ± 5.6	69.6 ± 5.4	<0.001
$SatO_2$ (%)	87.6 ± 7	96.9 ± 1.5	<0.001
ALT (IU/l)	28.5 ± 12.1	21.5 ± 6.1	0.007
GGTP (U/l)	75.7 ± 46.0	19.3 ± 9.6	<0.001
HA (ng/ml)	65.8 ± 59.3	27.0 ± 23.3	<0.001
ALP (U/l)	67.4 ± 21.2	51.2 ± 16.6	<0.001
Total protein (g/dl)	73.8 ± 8.9	74.1 ± 2.6	0.2
Albumin (g/dl)	49.4 ± 4.7	48.2 ± 5.8	0.4
Creatinine (μmol/l)	82.8 ± 15	77.1 ± 12.9	0.5
GFR (ml/min/m^2)	107.2 ± 17.6	109.3 ± 10.9	0.6
Cystatin C (mg/l)	1.0 ± 0.3	0.84 ± 0.1	0.01
Bilirubin (μmol/l)	21.7 ± 15.1	9.2 ± 4.6	<0.001
INR	1.2 ± 0.2	1 ± 0.1	<0.001
CRP (mg/l)	2.3 ± 2.2	2.0 ± 1.6	0.4
Uric acid (μmol/l)	371.3 ± 161.4	312.9 ± 96	0.09
NT-proBNP (pg/ml)	320.1 ± 544	54.3 ± 26.9	0.009
VO_{2peak} (ml/kg/min)	23.7 ± 6.4	49.3 ± 8.4	<0.001
VO_{2peak} (%N)	61.4 ± 16.8	121.6 ± 19.6	<0.001
VO_{2AT} (%)	46.2 ± 16.2	55.7 ± 7	0.005
T (min)	15.3 ± 2.7	17 ± 2.5	0.01

BMI, body mass index; SVEF, single ventricle ejection fraction; $SatO_2$, oxygen saturation; ALT, alanine aminotransferase; GGTP, gamma-glutamyltranspeptidase; HA, hyaluronic acid; ALP, alkaline phosphatase; GFR, glomerular filtration rate; INR, international normalized ratio; CRP, C-reactive protein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VO_{2peak}, peak oxygen uptake; VO_{2AT}, oxygen uptake at anaerobic threshold; *T*, exercise time.

Iron metabolism

When compared to the controls, the Fontan patients had higher UIBC (46.1 ± 12.6 μmol/l vs. 38.4 ± 11.9 μmol/l, $p = 0.02$), TIBC (62.8 ± 9.8 μmol/l vs. 57.8 ± 8.5 μmol/l, $p = 0.04$) and lower TSAT (27.4 ± 11.4% vs. 34.6 ± 13.4%, $p = 0.03$) (Table 2). There were no differences in iron and ferritin level between the groups. Iron level correlated positively with TSAT ($r = 0.89$, $p < 0.001$), MCV ($r = 0.45$, $p = 0.01$), MCH ($r = 0.53$, $p = 0.002$) and negatively with NYHA class ($r = -0.49$, $p = 0.03$) and RDW ($r = -0.49$, $p = 0.05$). Ferritin showed a positive association with WBC ($r = 0.64$, $p < 0.001$), HGB ($r = 0.7$, $p = 0.007$), MCHC ($r = 0.58$, $p < 0.001$), ALT ($r = 0.61$,

Table 2
Hematological and iron-related parameters in Fontan patients and controls.

Variable	Fontan $N = 32$	Controls $N = 30$	p
WBC (10^3/μl)	5.3 ± 1.6	5.8 ± 1.3	0.1
RBC (10^9/μl)	6.0 ± 2.1	4.8 ± 0.4	<0.001
Hg (g/dl)	16.7 ± 1.4	14.2 ± 1.3	<0.001
HCT (%)	49 ± 3.4	42.1 ± 3.1	<0.001
MCV (fL)	84.8 ± 11.6	87.2 ± 3.0	0.4
MCH (fL)	29.6 ± 1.7	29.4 ± 1.3	0.7
MCHC (mmol/l)	33.1 ± 5.5	33.7 ± 1.4	0.3
RDW (%)	14.3 ± 2.4	12.8 ± 0.5	<0.001
Platelet count (10^3/μl)	131.9 ± 36.8	224.5 ± 48.3	<0.001
Iron (μmol/l)	16.7 ± 6.4	19.4 ± 6.5	0.1
Ferritin (μg/ml)	112.6 ± 78.6	86.6 ± 59.1	0.1
UIBC (μmol/l)	46.1 ± 12.6	38.4 ± 11.9	0.02
TIBC (μmol/l)	62.8 ± 9.8	57.8 ± 8.5	0.04
TSAT (%)	27.4 ± 11.4	34.6 ± 13.4	0.03

WBC, white blood cells; RBC, red blood cells; Hg, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; UIBC, unsaturated iron binding capacity; TIBC, total iron binding capacity; TSAT, transferrin saturation.

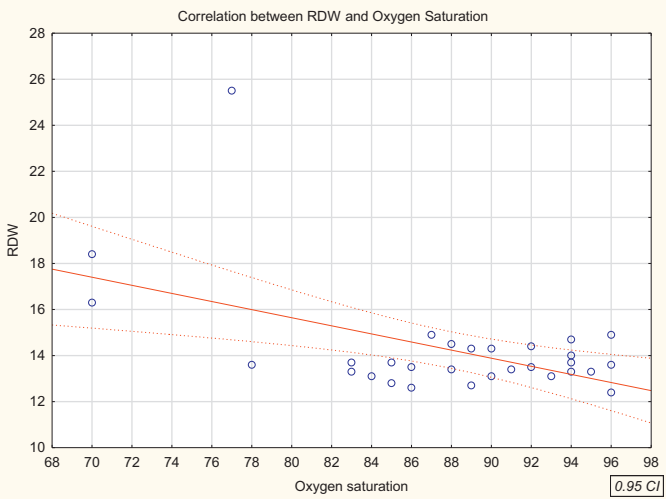

Fig. 1. Correlation between red cell distribution width (RDW) and oxygen saturation in Fontan patients.

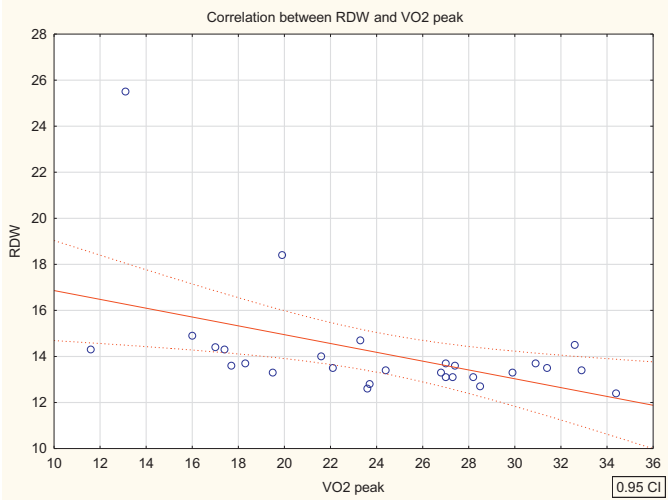

Fig. 2. Correlation between red cell distribution width (RDW) and oxygen uptake (VO_2 peak) in Fontan patients.

$p < 0.001$), TSAT ($r = 0.42$, $p = 0.02$), and GGTP ($r = 0.4$, $p < 0.04$) and a negative association with TIBC ($r = -0.55$, $p = 0.001$) and UIBC ($r = -0.54$, $p = 0.002$). The results are shown in Fig. 3. Univariate analysis for RDW, VO_{2peak}, and ferritin level is shown in Table 3.

In the group of patients with RDW above 14.5%, compared to patients with normal range, significantly lower values of $SatO_2$ ($83.1 \pm 10.8\%$ vs. $89.3 \pm 4.8\%$; $p = 0.04$) and iron (12.2 ± 4.6 μmol/l vs. 18.5 ± 5.9 μmol/l; $p = 0.02$) were observed (Fig. 4). We did not note any differences in hematological and iron-related parameters in the groups of Fontan patients taking vs. not taking warfarin (data not shown).

To determine the independent effect of clinical and laboratory variables, the stepwise regression analysis for RDW, VO_{2peak}, and ferritin as dependent variables was performed. The multivariate model showed that $SatO_2$ ($\beta = -0.29$, $p = 0.03$) and cystatin C ($\beta = 0.65$, $p < 0.001$) were the independent predictors of RDW ($R^2 = 0.6$, $p < 0.001$). Interestingly, ALT ($\beta = 0.44$, $p = 0.01$) was an independent predictor of ferritin level ($R^2 = 0.36$, $p = 0.007$). RDW was an independent predictor of oxygen uptake ($R^2 = -0.39$, $p = 0.008$).

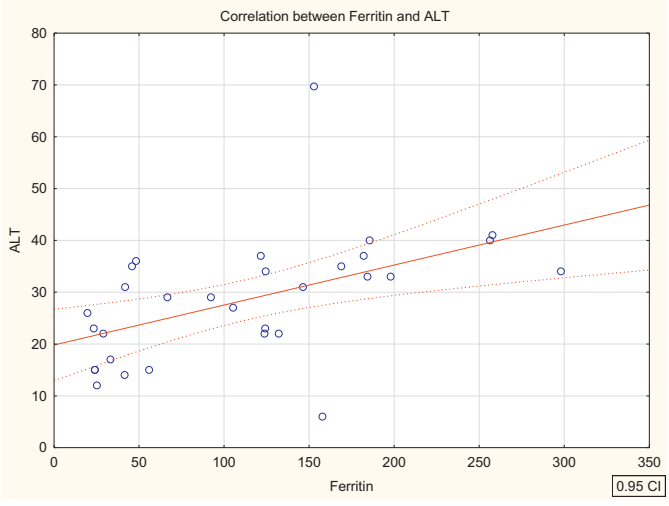

Fig. 3. Correlation between alanine aminotransferase (ALT) and ferritin levels in Fontan patients.

Table 3
Univariate analysis for RDW, VO_{2peak}, and ferritin level.

Variables	RDW		VO_{2peak}		Ferritin	
	β	p	β	p	β	p
Age (years)	0.3	0.1	−0.29	0.1	0.06	0.8
SVEF (%)	−0.44	0.01	0.45	0.01	0.09	0.6
$SatO_2$ (%)	−0.6	<0.001	0.27	0.2	0.31	0.09
ALT (IU/l)	−0.33	0.07	0.28	0.1	0.5	0.004
GGTP (U/l)	−0.15	0.5	0.05	0.8	0.43	0.03
HA (ng/ml)	0.45	0.01	−0.4	0.03	0.29	0.1
ALP (U/l)	0.37	0.04	−0.37	0.04	0.1	0.06
Total protein (g/dl)	−0.1	0.6	0.2	0.3	0.23	0.2
Albumin (g/dl)	−0.35	0.05	0.43	0.02	0.26	0.2
Creatinine (μmol/l)	0.3	0.05	−0.15	0.5	0.26	0.2
eGFR (ml/min/m^2)	−0.6	<0.001	0.37	0.6	0.1	0.6
Cystatin C (mg/l)	0.75	<0.001	−0.42	0.02	−0.28	0.1
Bilirubin (μmol/l)	0.01	0.9	0.3	0.1	−0.004	0.9
INR	0.1	0.6	−0.35	0.07	−0.05	0.8
CRP (mg/l)	0.27	0.2	−0.3	0.2	−0.17	0.4
Uric acid (μmol/l)	0.56	0.001	−0.37	0.05	−0.22	0.2
NT-proBNP (pg/ml)	0.27	0.2	0.04	0.9	0.08	0.7
VO_{2peak} (ml/kg/min)	−0.48	0.01			0.1	0.6
VO_{2peak} (%N)	−0.53	0.007			0.2	0.4
VO_{2AT} (%)	−0.31	0.1	0.48	0.009	0.28	0.1
T (min)	−0.58	0.001	0.8	<0.001	0.1	0.6
WBC (10^3/μl)	−0.45	0.01	0.09	0.6	0.46	0.01
RBC (10^9/μl)	−0.58	0.001	0.1	0.6	0.04	0.8
Hg (g/dl)	−0.22	0.2	−0.1	0.6	0.47	0.006
HCT (%)	−0.07	0.7	−0.31	0.1	0.35	0.5
MCV (fL)	−0.58	0.001	0.38	0.04	0.21	0.3
MCH (fL)	−0.36	0.05	0.41	0.03	0.32	0.08
MCHC (mmol/l)	−0.14	0.5	0.35	0.07	0.26	0.2
RDW (%)			−0.48	0.01	−0.33	0.07
Platelet count (10^3/μl)	0.07	0.7	−0.07	0.7	0.14	0.4
Iron (μmol/l)	−0.4	0.03	0.22	0.3	0.4	0.03
Ferritin (μg/ml)	−0.33	0.07	0.1	0.6		
UIBC (μmol/l)	0.38	0.4	−0.24	0.2	−0.43	0.02
TIBC (μmol/l)	0.21	0.3	−0.16	0.4	−0.42	0.02
TSAT (%)	−0.38	0.04	0.21	0.3	0.4	0.01
Postoperative time (years)	0.4	0.03	−0.19	0.3	0.2	0.3
NYHA	0.38	0.04	−0.42	0.02	−0.34	0.06

SVEF, single ventricle ejection fraction; $SatO_2$, oxygen saturation; ALT, alanine aminotransferase; GGTP, gamma-glutamyltranspeptidase; HA, hyaluronic acid; ALP, alkaline phosphatase; eGFR, estimated glomerular filtration rate; INR, international normalized ratio; CRP, C-reactive protein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VO_{2peak}, peak oxygen uptake; VO_{2AT}, oxygen uptake at anaerobic threshold; T, exercise time; WBC, white blood cells; RBC, red blood cells; Hg, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; UIBC, unsaturated iron binding capacity; TIBC, total iron binding capacity; TSAT, transferrin saturation; NYHA, New York Heart Association.

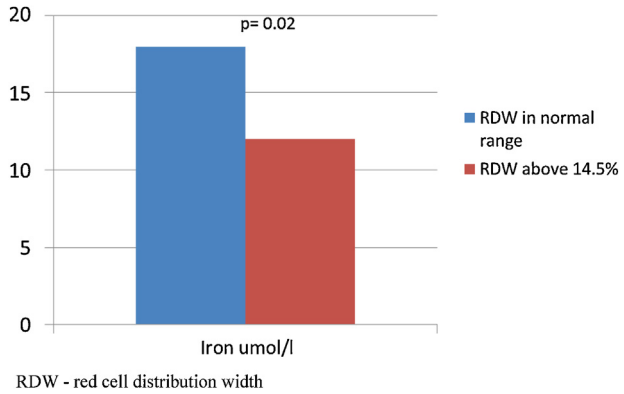

Fig. 4. Iron levels in Fontan patients with red cell distribution width (RDW) in normal range and with RDW above 14.5%.

Discussion

In the present study we demonstrate the changes in hematological and iron-related parameters in adult patients late after Fontan operation. We confirm higher RBC count, RDW, HGB, and HCT levels and iron deficiency as evidenced by increased levels of TIBC and UIBC, and decreased TSAT compared to the controls. Furthermore, we present an association between RDW and exercise tolerance in adult Fontan patients.

The therapeutic goal of the Fontan procedure is the normalization of the volume load of the single ventricle and separation of the pulmonary and systemic circulation, thus achieving normal or near-normal blood oxygenation [2]. Compared to the controls, the Fontan patients demonstrated significantly lower arterial blood saturation. It may result from a progressive drop in single ventricle function, a rise in pulmonary pressure, and right-to-left shunt through an atrial fenestration or venovenous collaterals [3]. In the presented group, the Fontan patients showed a significantly impaired systolic function of the systemic ventricle when compared to the control subjects. A patent fenestration was present in 30% of the patients. As a result of chronic tissue hypoxia, erythropoietin production increases, which leads to an increase in the number of red blood cells and secondary polyglobulia. However, we did not find the correlation between HGB and oxygen saturation. Broberg et al. [6] demonstrated that nonoptimal relation between these variables may result from inadequate erythropoiesis in patients with cyanotic congenital heart diseases. Iron, foliate, or vitamin B_{12} deficiency, increased erythropoietin or a right-shifted oxygen-HGB curve might alert this relation [6,10]. Anemia is common in cohorts of adult patients with cyanotic congenital heart diseases, ranging from 25% to 60%, depending on the study [9,11,13]. The cause of anemia in this group of patients is multifactorial and unclear [11,14–16]. Importantly, in the present study, we have not diagnosed anemia in the Fontan patients. In the earlier investigation, Collins et al. [11] detected anemia in 48% of adult Fontan patients (mean age 34.8 ± 8 years). In the above report, hyponatremia, a decreased renal function, and the use of warfarin were the independent predictors of anemia. In our study, the patients were younger (mean age 24.9 ± 6 years) in relation to the studies reported previously [7,11]. The risk of anemia development increases with deteriorating renal function [15,16]. In the present study, we have not observed differences in creatinine concentrations; however, cystatin C level was higher in the Fontan group.

Although anticoagulation increases the risk of blood loss, we also have not noted a history of chronic hemorrhage and we have not found any differences in hematological and iron-related parameters in the groups of patients taking vs. not taking warfarin.

The most common cause of anemia in patients with congenital heart diseases is iron deficiency [10,14]. In the present study, no differences in serum iron levels in the Fontan patients compared to the controls were observed. The literature on the subject lacks data on serum iron levels in adult Fontan patients. Yetman and Everitt [17] describe the case of an adult Fontan patient with iron deficiency anemia who developed protein-losing enteropathy. Following intravenous iron administration, the hematological parameters improved and symptoms associated with protein-losing enteropathy diminished. In the present study, low iron values were noted in four (13%) patients, of which two (6%) presented with symptoms of protein-losing enteropathy, manifested as edema of the lower extremities and ascites. Moreover, low iron level noted in the investigated group was associated with more intensified symptoms of heart failure. Iron concentration undergoes daily variations and shows considerable individual biological versatility, which makes it difficult to assess iron metabolism. In the present study, we demonstrated iron deficiency in the Fontan patients compared to the control group. There was a significantly higher total iron binding and unsaturated iron binding capacity, as well as decreased transferrin saturation. Ferritin is the major iron-storing protein. However, a well-known limitation is that it is also an acute-phase protein and its level may be increased in infective or inflammatory diseases independently from the iron status [10]. In our study, the principal factor determining the level of ferritin in Fontan patients was ALT level. Kowdley et al. [18] demonstrated that an increased level of ferritin concentration ($>1.5\times$ upper limit of the normal value) is an independent index of liver damage and should be applied as a useful non-invasive test identifying individuals at risk of nonalcoholic steatohepatitis and high-grade fibrosis. Similarly, Wojtowicz-Chomicz et al. [19] noted high ferritin values in patients with nonalcoholic steatohepatitis and alcoholic liver cirrhosis. The analyzed group of Fontan patients demonstrated signs of liver damage, conforming to earlier reports [20,21]. The major factor that causes liver failure in Fontan patients is long-term hepatic venous congestion. Camposilvan et al. [22] reported that hepatic abnormalities were found in up to 53% of Fontan patients.

RDW defines the degree of anisocytosis. It is particularly useful in diagnosing the early phase of anemia, when the value of MCV is still normal [9]. A recent study has described elevated RDW in patients with heart failure, resulting from multiple conditions that culminated in ineffective erythropoiesis or red blood cells destruction [23]. Emans et al. [24] confirmed that RDW is associated with heart failure events in an apparently healthy population. Furthermore, high RDW levels were associated with physical inactivity in this group. In the present study, the Fontan patients have a high value of RDW. Moreover, peripheral oxygen saturation and cystatin C level were an independent determinant of RDW. This is in agreement with a previous study showing an ineffective erythropoiesis and impaired renal function as determinants of high RDW in heart failure [25]. High RDW values were also associated with low iron levels. One possible explanation to this finding is that RDW might reflect the disease's severity associated with (i) surgery-related iron loss, (ii) reduced gastrointestinal iron uptake, and (iii) increased gastrointestinal iron loss associated with protein-losing enteropathy [5,11,17]. Similarly, Martinez-Quintana and Rodriguez-Gonzalez [9] demonstrated that RDW is an inexpensive, easy to determine parameter, which allows the diagnosis of early phases of iron deficiency in patients with congenital cyanotic heart defects. Peak oxygen consumption is considered as the gold standard to assess exercise performance. In our study, peak oxygen uptake and the duration of CPET in Fontan patients were significantly decreased. Diminished exercise capacity was associated with an early start of anaerobic metabolism. Moreover, RDW was an independent predictor of diminished exercise capacity in adult Fontan patients. Van Craenenbroeck et al. [23] demonstrated that RDW was associated with lower exercise capacity in patients with heart failure independent of other widely accepted determinants such as age, HGB, renal function, and NT-proBNP values. The mechanism by which RDW is associated with impaired exercise capacity is multifactorial and poorly understood. One might expect that RDW reflects disease severity and might not be causal. However, low oxygen saturation may lead to inadequate erythropoiesis, resulting in RDW increase, which in turn may decrease oxygen supply to the muscles [24].

The results discussed above may have important implications for the treatment of Fontan patients. However, more extensive studies with a higher number of subjects are needed to determine iron deficiency in this population. The influence of the potentially beneficial effects of iron supplementation on the clinical status, the course of the disease, and the prognosis of Fontan patients should be addressed in future prospective clinical trials [26].

Several limitations of the study should be acknowledged. First, the number of the patients in the study was small and heterogeneous with respect to cardiac diagnosis and Fontan surgery type.

Hypochromia and microcytosis could have been masked by other factors, such as vitamin B_{12} and/or foliate deficiency [14]. We have not measured vitamin B_{12}, foliate, homocysteine, and erythropoietin, which can be considered as another limitation of the study.

Conclusion

In the present study we show that in adult patients after Fontan operation, despite having increased HGB, HCT, and red blood cells, insufficient iron stores were detected. RDW is an indicator of iron deficiency in those patients and correlates with lower physical activity. Elevated ferritin levels in adult patients after Fontan surgery are associated with liver failure.

Conflict of interest

None declared.

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References

[1] Fontan F, Baudet E. Surgical repair of tricuspid atresia. Thorax 1971;26:240.
[2] Khairy P, Poirier N, Mercier LA. Univentricular heart. Circulation 2007;115:800–12.
[3] Atz AM, Travison TG, McCrindle BW, Mahony L, Quartermain M, Williams RV, Breitbart RE, Lu M, Radojewski E, Margossian R, Covitz W, Gersony WM, Pediatric Heart Network Investigators. Late status of Fontan patients with persistent surgical fenestration. J Am Coll Cardiol 2011;57:2437–43.
[4] Tomkiewicz-Pajak L, Hoffman P, Trojnarska O, Bednarek J, Płazak W, Pajak J, Olszowska M, Komar M, Podolec P. Long term follow-up in adult patients after Fontan operations. Polish J Cardiothorac Surg 2013;10:357–63.
[5] Goldberg DJ, Dodds K, Rychik J. Rare problems associated with the Fontan circulation. Cardiol Young 2010;20(Suppl. 3):113–9.
[6] Broberg CS, Jayaweera AR, Diller GP, Prasad SK, Thein SL, Bax BE, Burman J, Gatzoulis MA. Seeking optimal relation between oxygen saturation and hemoglobin concentration in adults with cyanosis from congenital heart disease. Am J Cardiol 2011;107:595–9.
[7] Nanas JN, Matsouka Ch, Karageorgopou D, Leonti A, Tsolakis E, Drakos SG, Tsalgalou EP, Maroulidis GD, Aleksopoulos GP, Kanakakis JE, Anastasiou-Nana MI. Etiology of anemia in patients with advanced heart failure. J Am Coll Cardiol 2006;48:2485–9.
[8] Pasricha SR, Drakesmith H, Black J, Hipgrave D, Biggs BA. Control of iron deficiency anemia in low- and middle-income countries. Blood 2013;121:2607–17.
[9] Martinez-Quintana E, Rodriguez-Gonzalez F. Iron deficiency anemia detection from hematology parameters in adult congenital heart disease patients. Congenit Heart Dis 2013;8:117–23.
[10] Dopsaj V, Mikovic-Golubovic G, Martinovic J, Kalimanowska-Ost D. Evaluation of derived Coulter red blood cell parameters for the assessment of iron deficiency in adults with congenital heart disease. Int J Lab Hematol 2012;34:461–72.
[11] Collins N, Piran S, Harrison J, Azevedo E, Oechslin E, Silversides CK. Prevalence and determinants of anemia in adults with complex congenital heart disease and ventricular dysfunction (subaortic right ventricle and single ventricle physiology). Am J Cardiol 2008;102:625–8.
[12] Van De Bruaene A, Delcroix M, Pasquet A, De Backer J, De Pauw M, Naeije R, Vachiéry JL, Paelinck B, Morissens M, Budts W. Iron deficiency is associated with adverse outcome in Eisenmenger patients. Eur Heart J 2011;32:2790–9.
[13] Jensen AS, Johansson PI, Idorn L, Sorensen KE, Thilen U, Nagy E, Furenas E, Sondergaard L. The hematocrit – an important factor causing impaired haemostasis in patients with cyanotic congenital heart disease. Int J Cardiol 2013;167:1317–21.
[14] Kaemmerer H, Fratz S, Braun SL, Koelling K, Eicken A, Brodherr-Heberlein S, Pietrzik K, Hess J. Erythrocyte indexes, iron metabolism, and hyperhomocysteinemia in adults with cyanotic congenital cardiac disease. Am J Cardiol 2004;94:825–8.
[15] Wastenbrink BD, Visser FW, Voors AA, Smilde TDJ, Lipsic E, Navis G, Hillege HL, van Gilst WH, van Veldhuisen DJ. Anaemia in chronic heart failure is not only related to impaired renal perfusion and blunted erythropoietin production, but to fluid retention as well. Eur Heart J 2007;28:166–71.
[16] Dimopoulos K, Diller GP, Koltsida E, Pijuan-Domenech A, Papadopoulou SA, Babu-Narayan SV, Salukhe TV, Piepoli MF, Poole-Wilson FA, Best N, Francis DP, Gatzoulis MA. Prevalence, predictors, and prognostic value of renal dysfunction in adults with congenital heart disease. Circulation 2008;117:2320–8.
[17] Yetman AT, Everitt MD. The role of iron deficiency in protein-losing entheropathy following the Fontan procedure. Cong Heart Dis 2011;6:370–3.
[18] Kowdley KV, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, Sanyal AJ, Nelson JE, NASH Clinical Research Network. Serum ferritin is an independent predictor of histological severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2012;55:77–85.
[19] Wojtowicz-Chomicz K, Cichoz-Lach H, Lis E, Kowalik A, Słomka M, Celinski K. Serum iron parameters in chronic liver diseases. Pol Merkur Lekarski 2013;35:77–81.
[20] Oka T, Kato R, Fumino S, Toiyama K, Yamagishi M, Itoi T, Hamaoka K. Non-invasive estimation of central venous pressure after Fontan procedure using biochemical markers and abdominal echography. J Thorac Cardiovasc Surg 2013;146:153–7.
[21] Hayashi T, Inuzuka R, Shindo T, Hirata Y, Shimizu N, Oka A. Serum hyaluronic acid concentration in Fontan circulation: correlation with hepatic function and portal vein hemodynamic. Pediatr Cardiol 2013;(October) [Epub ahead of print].
[22] Camposilvan S, Milanesi O, Stellin G, Pettenazzo A, Zancan L, D'Antiga L. Liver and cardiac function in the long term after Fontan operation. Ann Thorac Surg 2008;86:177–82.
[23] Van Craenenbroeck EM, Pelle AJ, Beckers PJ, Possemiers NM, Ramakers C, Vrints CJ, Van Hool V, Denollet J, Conraads VM. Red cell distribution width as a marker of impaired exercise tolerance in patients with heart failure. Eur J Heart Fail 2012;14:54–60.
[24] Emans EM, Gaillard CAJM, Pfister R, Tanck MW, Boekholdt SM, Wareham NJ, Khaw KT. Red cell distribution width is associated with physical inactivity and heart failure independent of establish risk factors, inflammation or iron metabolism; the EPIC-Norfolk study. Int J Cardiol 2013;168:3550–5.
[25] Förhécz Z, Gombos T, Borgulya G, Pozsonyi Z, Prohászka Z, Jánoskuti L. Red cell distribution width in heart failure: prediction of clinical events and relationship with markers of ineffective erythropoiesis, inflammation, renal function, and nutritional state. Am Heart J 2009;158:659–66.
[26] Tay ELW, Peset A, Papaphylactou M, Inuzuka R, Alonso-Gonzalez R, Giannakoulas G, Tzifa A, Goletto S, Broberg C, Dimopoulos K, Gatzoulis MA. Replacement therapy for iron deficiency improves exercise capacity and quality of life in patients with cyanotic congenital heart disease and/or the Eisenmenger syndrome. Int J Cardiol 2011;151:307–12.